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14. ABSTRACT The Prostate Cancer Pathology Resource Network (which has since been renamed the Prostate Cancer Biorepository Network or PCBN) is a collaboration between the Johns Hopkins School of Medicine (JHU) and the New York University School of Medicine (NYU). The goal of the PCBN is to develop a biorepository with high quality, well annotated specimens that can be used by prostate cancer researchers. The specimens in the PCBN include prostatectomy tissues (frozen, paraffin embedded, and tissue microarrays (TMAs), serum, plasma, buffy coat, prostatic fluid, and derived specimens (DNA and RNA); these specimens are linked to clinical and outcome data and supported by an informatics infrastructure that will eventually be grid-enabled through caTISSUE. The PCBN is currently made accessible to outside researchers through a website. The PCBN has been open to researchers since July 1 2011. Since that time it has been publicized at national and international meetings, surveyed prostate cancer researchers across the country for their tissue and biospecimen needs, finalized policies and procedures for access to tissue, finalized SOPs for most common processes, markedly revised the website to include improved forms, descriptions of procedures, SOPs and biospecimen resources, conducted biospecimen research on DNA and RNA best practices and presented these at international meetings, significantly increased the number of TMAs and specimens available, and fulfilled 6 tissue requests with 9 more in process, from researchers in the US and abroad.				
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INTRODUCTION

The Prostate Cancer Biorepository Network (PCBN) is a collaboration between the Johns Hopkins School of Medicine (JHU), the New York University School of Medicine (NYU), and the Department of Defense (DOD).

The PCBN is organized with a **Coordinating Center** (JHU – led by Bruce Trock, Ph.D.), and **Network Sites** at NYU (led by Jonathan Melamed, M.D. and Peng Lee, M.D.) and JHU (led by Angelo De Marzo, M.D. and George Netto, M.D.). **The goal of the PCBN** is to develop a biorepository with high quality, well-annotated specimens obtained in a systematic, reproducible fashion using optimized and standardized protocols, and an infrastructure to facilitate the growth of the resource and its wide usage by the prostate cancer research community. The specimens in the PCBN include tissues from prostatectomies, serum, plasma, buffy coat, prostatic fluid, derived specimens such as DNA and RNA, linked to clinical and outcome data, and supported by an informatics infrastructure with the capability to deposit data into caTISSUE. A website has been established to make the PCBN accessible to the prostate cancer research community: <http://prostatebiorepository.org>

The current report reviews the history of the PCBN to establish context, and documents progress to date, with emphasis on the most recent 12 months, and also addresses issues raised at the last EAB meeting in September 2011.

BODY

History

The original intent of the Program Announcement for the Prostate Cancer Pathology Resource Network Award was a pilot effort to establish infrastructure and a nascent biorepository that, at the end of the 3 year funding period would be positioned to function as a full-fledged biorepository with support for continued operation expected to be derived from renewal funding, other grant sources, institutional support, or some combination thereof. Although originally intended as a network comprised of 3 sites (the institution that was awarded the Coordinating Center would function in that capacity and as a network site), only 2 institutions were funded.

The criteria for evaluating the Network comprised the following 6 **Performance Metrics** described in the PA (detailed descriptive text from some metrics not included):

- The Network Coordinating Center must develop standard operating procedures for biospecimen collection methods and post-collection processing
- The Network Coordinating Center must demonstrate sufficient data quality control and assurance through documentation that standard operating procedures are being followed for biospecimen annotation (e.g., patient history and demographic, clinical history, treatment, pathology, and outcome such as disease progression, recurrence, and prostate specific antigen (PSA) levels and/or other biochemical status).
- The Network Coordinating Center must demonstrate sufficient and ongoing efforts to harmonize the biorepository informatics system with the informatics systems of other national biorepositories, including caBIG.

- Each Pathology Resource Network Site must contribute biospecimens from a minimum of 50 patients per year, with the expectation that biospecimen contribution will exceed the minimum requirement. Biospecimens from ethnic minority populations should match or exceed the existing ethnic minority patient population available to the Pathology Resource Network Site.
- Each Pathology Resource Network Site must submit quality data and reports in a timely manner as outlined by the Coordinating Center.
- Network Coordinating Center must demonstrate sufficient activity with the prostate cancer research community through ongoing documentation of Letters of Intent for utilization of biorepository specimens, to include the number of requests received, approved, or rejected, and the types of specimens distributed.

Discussion of progress will primarily center around these metrics, although we will also discuss other areas related to establishing the infrastructure, including governance and regulatory aspects. We also describe how we have addressed key personnel changes at each institution.

Progress

Progress will be described in 5 areas: policies for specimen access, informatics, biospecimens, standard operating procedures (SOPs), and marketing and usage by the research community.

Planning and initial formulation of Governance policies were described in the previous Annual Report. Since that time we have had additional face-to-face meetings for planning and problem solving in August 2011 (at JHU), and May 2012 (at NYU). *At the Sept 7 2011 meeting with the External Advisory Board (EAB)* it was recommended that we convene a Scientific Advisory Board separate from the Steering Committee (SC) to provide independent scientific guidance; Dr. Grizzle was recommended to be one of the members. That board has been convened and includes Dr. Grizzle, Dr. Scott Lucia (Pathologist, University of Colorado), and Dr. Daniel Lin (Urologist, Fred Hutchinson Cancer Research Center).

1. Policies for Specimen Access

These have been refined since the previous Annual Report. The SC developed **3 categories of specimens** that reflect a prioritization according to their rarity/research value:

Priority 1 specimens:

Specimens that are readily available, and which have little or no linked clinical data. These specimens will be made available for early stage research, e.g. to demonstrate that a particular biomarker is differentially expressed in normal vs. tumor tissue. *Little or no preliminary data will be required to justify the request.*

Priority 2 specimens:

Specimens that have greater research value, either due to their relative abundance or the richness of linked data or other linked specimen types. *Access to these specimens would require preliminary data showing*

that the biomarker assay performed well and that the biomarker was differentially expressed in cancer vs. normal.

Priority 3 specimens:

Rare and/or data-rich specimens. Requests for these specimens would *require more mature preliminary data, e.g. demonstration that the biomarker was correlated with a measure of aggressiveness to justify request for matched recurrent vs. non-recurrent cases.*

Review criteria for specimens are linked to these 3 priority categories. Requests for specimens of any type must meet the following requirements:

1. Scientifically valid objective
2. PI and institution have suitable experience and resources to conduct the study.
3. Methods and sample amount/number requested are reasonable.

In addition to these blanket requirements, requests for Priority 2 and 3 specimens require preliminary data as indicated in the italicized text above.

We have operationalized these definitions and criteria and posted them on the website. Requests for samples are first reviewed by Dr. Sfanos or Dr. Trock to determine that the necessary information is provided, the requested samples are available, and the request appears reasonable. The requests are then forwarded for review by the Scientific Advisory Board with Jonathan Melamed included to represent the PCBN (***this role for the SAB was also recommended by the EAB at the Sept. 7, 2011 meeting.***

1a. Specimens requiring collaboration. It is important to note that many of the previously existing specimens/data were developed by non-PCBN investigators at JHU and NYU, or with funds from other sources. For such resources that are particularly valuable or were very labor intensive to develop, the specimens may be made available to users in the form of a collaboration with the originator, rather than providing the samples without restriction. These primarily refer to 4 TMAs (see Section 3a: Biospecimens – TMAs). Collaboration may also be required for relatively large sample sets, i.e. requests for individual specimens (e.g. serum samples, DNA samples) from ≥ 200 cases. For such a request the PCBN would have a major role in the success of the project that would involve, among other things, a key contribution to study design, and thus a PCBN collaborator would be appropriate. For resources developed with significant effort and resources outside of PCBN the collaborator may be a non-PCBN investigator who is a stakeholder in the particular specimen set. Specimens requiring collaboration are so designated on the website.

2. Informatics

caTISSUE Suite was initially chosen as a common export format for both sites. Rather than deploying caTISSUE as the sole system it was decided to use it as a secondary system to which both JHU and NYU could send data to meet the requirements of the Award (and also to satisfy IRB requirements for de-identification). Both institutions have mapped data elements to caTISSUE and can automatically export their data to a caTISSUE-accessible format. Currently neither institution is planning to use caTISSUE as their primary informatics platform (in fact, one of the Scientific Advisory Board members, Dr. Grizzle, strongly argued

against it). NYU is moving to an enterprise-wide instance of LabVantage, and is using a RedCap system in the interim. JHU currently has a private instance of caTISSUE for PCBN, and the university is setting up an enterprise-wide caTISSUE instance. However, for most PCBN functions, we will continue to use TMAJ, which stores pathology and specimen data, and our Master Radical Prostatectomy database, which stores clinical and outcome data. Numerous projects over many years have shown that these 2 databases can be easily linked to query or output all data types necessary for PCBN.

In the previous report we described beginning the process of collecting data to annotate specimens with respect to pre-analytical variation. This data collection process is now fully active. Currently at JHU we are collecting the following annotation variables from >90% of cases: (1) time of incision start / incision close (as surrogate for time of devascularization), (2) the time when surgical pathology is paged to get the specimen, and (3) the time the specimen is frozen or placed in fixative. At NYU we are collecting the following annotation variables from most cases:

- time of incision start / incision close
- time of pedicle division
- time specimen in endocatch bag (robotic specimens)
- time specimen in specimen jar
- time specimen collected from OR
- time specimen grossed / subsequent freezing
- method of freezing
- specimen in OCT (yes/no)
- time specimen placed in formalin
- time of formalin fixation

2a. Website. The website www.prostatebiorepository.org was made available to the public in June 2011, and during the current reporting period has been significantly updated. It includes the following (“*” indicates feature has been updated or added anew since the last Annual Report):

- a description of the PCBN
- a listing of the people involved
- information regarding PCBN governance*
- listings and descriptions of available specimens and those in development*
- policies, requirements, prioritization scheme and review criteria for specimens*
- cost recovery schedule*
- updated application forms with instructions and automatic “submit” feature*
- SOPs used in PCBN*
- FAQs*
- useful links to other websites
- a query feature that automatically directs questions to the entire PCBN team
- biospecimen science activities including links to posters and abstracts*

Since its inception the website has had nearly 2000 visits by over 600 individuals, with over 7000 page views. **Appendix 1** shows the pattern of website traffic during the period November 2011-May 2012, annotated to

show specific events that influenced traffic (e.g. PCBN survey sent to Prostate Cancer Foundation members, AACR, etc – see section on Marketing and Usage for further details on these activities).

3. Biospecimens:

One of the strengths of both the NYU and JHU sites is the large number and variety of biospecimens, both in existing archives and those newly available due to large patient volumes. In particular, both teams have extensive experience building and sharing biospecimens in the form of TMAs. Other specimens include fixed tissue (radical prostatectomy, TURP, suprapubic prostatectomy), snap frozen tissue (radical prostatectomy, seminal vesicles), body fluids (serum, plasma, buffy coat, prostatic fluid; most can be matched to tumor and benign tissue), and derived specimens (DNA, RNA, protein). The table below shows the total specimens newly accrued to the PCBN since inception, and during the last 12 months:

Specimen Category	Total Since Start of Funding (JHU Total / NYU Total)	Last 12 months (JHU / NYU)
Total cases accrued	2002 (1512 / 490)	1169 (850 / 319)
Frozen tissue cases	459 (220 / 239)	252 (99 / 153)
Seminal vesicle cases	1535 (1296 / 239)	798 (645 / 153)
Prostatic fluid cases	1219 (1219 / 0)	623 (623 / 0)
Seminal vesicle fluid cases	116 (0 / 116)	90 (0 / 90)
Metastatic cases	120 (0 / 120)	39 (0 / 39)

5a. TMAs. TMAs currently available to the PCBN and those in development are shown in the table below, along with the Priority level (3 being the most valuable). The table also indicates which TMAs are made available in the form of a collaboration, and those which are newly developed for the PCBN (i.e. “prospective”).

TMAs Currently Available:

TMA	Description	Priority
8 Case Test	For use in testing an investigator's IHC assay for biomarker of interest on PCBN material or for determining the prevalence of candidate biomarker in tumor. Includes 8 cases tumor and normal, no clinical data, across 1 block.	1

40 Case Screening	For early phase comparison of tumor vs. normal. Includes 40 cases tumor and normal, 4 cores each, no clinical data, across 1 block.	1
80 Case Grade/Stage	For testing biomarkers with evidence of association with cancer. Includes range of Gleason grade & pathology stage. 80 cases and matched normal – 4 cores each, with limited clinical data, across 2 blocks.	2
200 Case Grade/Stage	For testing biomarkers strongly associated with cancer. Includes range of Gleason grade and pathology stage. 200 cases and matched normal – 4 cores each, with key clinical variables across 5 blocks.	2
237 Case Natural History of Prostate Cancer (Collaboration)	For testing biomarkers associated with the natural history of prostate cancer progression. Constructed from 237 cases from the “Pound Paper” (Pound et al. 1999 JAMA 281(17):1591-7), tumor and normal – 4 cores each with the cases, with key clinical variables across 6 blocks.	3
10 Case Test PSA Progression	For testing IHC assay before 726 case PSA Progression Array is released. Includes 10 cases with tumor (4 cores) and normal (4 cores), across 1 block.	1
726 Case PSA Progression (Collaboration)	For testing biomarkers associated with prostate cancer progression. Includes 726 cases tumor (4 cores) and normal (4 cores), with key clinical variables, across 16 blocks.	3
Lymph Node Mets (Collaboration)	Matched primary tumor and lymph node mets (hormone naive); 80 cases	3
150 Case Race Disparity (Collaboration) <i>(Prospective)</i>	For comparing biomarkers in African American and Caucasian patients. Includes 75 cases tumor and normal from each group matched on grade and stage; key clinical and demographic data across 4 blocks.	3
343 Case Family History	For testing biomarkers associated with hereditary risk of prostate cancer. Includes 343 cases matched positive & negative family history cases (matched on Gleason score) across 7 blocks.	2
56 Case Hormone Sensitivity	For testing biomarkers associated with androgen biology. Includes hormone naive vs. hormone refractory cases totalling 56 cases; 18 hormone resistant, 18 hormone naïve, 10 radical prostatectomy (RP) with neoadjuvant treatment, 10 RP without neoadjuvant treatment, 5 normal	2

	from RP with neoadjuvant treatment and 5 normal RP without neoadjuvant treatment – 4 cores per case, with key clinicopathological variables across 1 block.	
217 Case Biochemical Recurrence	For testing biomarkers strongly associated with known prognostic factors (e.g. stage, grade). Includes patients with vs. without biochemical recurrence. Total 217 cases, 23 with adjacent normal (4-5 tumor cores, 4 normal cores) and 13 BPH cases (4 cores), most with clinicopathological variables across 5 blocks.	3
50 Case Benign Prostatic Hyperplasia (BPH)	For testing biomarkers associated with benign prostatic hyperplasia (BPH). Includes 50 cases; 28 BPH from RP specimens, 12 BPH from suprapubic specimens, 10 normal from small prostate specimens, across 1 block.	1
119 Case High-Grade PIN	For testing biomarkers associated with high-grade prostatic intraepithelial neoplasia. Includes 119 cases, 4 cores HGPIN, 1 core tumor, with key clinical variables across 2 blocks.	2
Fixation (<i>Prospective</i>)	For evaluating the impact of variation in fixation time on biomarkers of interest. Includes 27 cases, 5 time points per case (4, 8, 12, 24 and 48hr in 10%NBF), with diagnostic block, no clinical data.	1
Ischemia / Fixation Delay (<i>Prospective</i>)	For evaluating the impact of variation in tissue processing time (delay to fixation) on biomarkers of interest. Includes 15 cases, 4 time points per case (0 1, 2, 4hr delay to fixation), with diagnostic block, no clinical data.	1

TMAs in Development :

TMA	Description	Priority
Gleason Grade (<i>Prospective</i>)	For evaluating biomarker associations with Gleason grade. Includes cases of tumor matched non-neoplastic tissue, with a range of primary and secondary Gleason grades; key clinical data	2
High Grade Recurrence (<i>Prospective</i>)	Gleason 8 tumors: cases with recurrence in <5 years matched to cases without recurrence for \geq 10 years	3

Race Disparity
(Collaboration)
(Prospective)

For comparing biomarkers in African American and Caucasian patients. Includes 150 cases tumor and normal from each group matched on grade and stage; key clinical and demographic data

3

For the TMAs in development, the complete Race Disparity TMA set is awaiting 50 African American cases and 50 matched Caucasian cases from NYU that have all been identified, blocks have been retrieved, and are in the process of extracting cores; they expect to be complete within 3 months. JHU has completed (see above) part of the Race Disparity TMA set. Many of the cases used in the Race Disparity TMA have matched frozen tissue from which DNA or RNA can be derived. We had hoped to include in this TMA 50 African American and 50 Caucasian cases from the DOD-funded Prostate Cancer Project Consortium, through a collaboration with James Mohler at Roswell Park. The terms of the collaboration and the MTA are still under discussion so our current plan is to add them at a later time when they become available.

5b. Autopsy and Advanced Disease Specimens. NYU has been approved by the IRB to start a warm autopsy program at both NYU and VA hospitals. Dr. Melamed has arranged for the necessary permissions and personnel to perform autopsies on an on-call basis. Currently, 7 men with advanced metastatic disease have signed consent agreeing to rapid autopsy in the event of their death. A similar program at JHU is being considered after a dormant period, although additional funds will be required due to the personnel demands of the program. The JHU program has collected tissue from 33 autopsies (prior to PCBN). These were the subject of some ownership issues with a previous investigator. Ownership has now been resolved and the specimens are being catalogued, with plans to develop TMAs for PCBN.

At JHU we are in the process of acquiring a large collection of serum samples (assembled and maintained by Dr. Mario Eisenberger - JHU) from men with biochemical recurrence or metastatic disease; serial samples are available for some men. Many of these can be matched to prostatectomy tissue. Most of these samples have not undergone any freeze-thaw cycles. We are in the process of transferring the database and do not yet have a breakdown of the number of cases or their clinical characteristics.

5c. Derived Specimens. In the past 12 months the following specimens have been extracted from frozen tissue at JHU:

DNA (70 samples from 32 cases)
RNA (86 samples from 44 cases)
Protein (30 samples from 17 cases)

Almost all samples also have matched tumor and benign tissue available. These samples were extracted following a comprehensive quality control process to determine the optimal protocol. ***All were obtained since funding began, thus "prospective."*** Because the protocols for RNA and DNA have been extensively optimized at JHU, the decision was made for NYU to send punch biopsies from frozen prostate tissues to JHU for derivative extraction, to maintain standardization. NYU will begin sending those specimens.

5d. Body Fluids. In addition to the serum samples from metastatic cases described above, we have a collection of serum, plasma, and buffy coat from nearly 350 men, collected at the time of radical prostatectomy and with clinical, pathology and follow-up data, with median follow-up time 3 years (range 1-8); **89 have been collected since the start of funding**. These can all be matched to tumor and benign tissue and also have extensive epidemiology and dietary data. An equal number of samples are available from men without known cancer (biopsy negative or PSA<2.5). We also have several thousand prostatic fluid samples manually expressed from *ex vivo* prostates at the time of surgery. Finally, we have just reached an agreement with Dr. Alan Partin (JHU) to make available 300 urine samples collected prior to biopsy (but post DRE) and frozen without centrifuging or buffer; approximately 100 are from men found to have cancer at biopsy.

4. Standard Operating Procedures (SOPs)

The SOPs at both sites for the major tissue-oriented processes (such as harvesting prostatectomy fresh frozen tissue, fixation and processing, extracting DNA, RNA and protein, and extracting serum, plasma and buffy coat) have all been posted on the PCBN website. *At the Sept 7 2011 EAB meeting* it was recommended that we perform due diligence to identify the most optimal protocols. SOPs for quality control of DNA and RNA have also been posted. The due diligence for DNA, RNA and protein protocols is included as **Appendix 2**. SOPs for harvesting fresh frozen tissue at both sites are identical, however protocols for fixation and processing are those that have been established on the basis of clinical considerations at each institution and do have some variations. In an effort to harmonize these protocols NYU has begun injecting formalin into radical prostatectomy specimens, and will begin microwave fixation. NYU has also begun performing p27 immunohistochemistry as an indicator of fixation quality of tissues to be used in TMAs.

The protocol for extracting serum, plasma and buffy coat from blood samples was provided by Dr. Hans Lilja (Memorial Sloan Kettering), one of the world's foremost authorities on blood-based biomarkers for prostate cancer. This protocol is also nearly identical to the EDRN protocol

(<http://edrn.nci.nih.gov/resources/standard-operating-procedures/standard-operating-procedures/serum-sop.pdf>). Prostatic fluid is also harvested by manual compression of *ex vivo* surgical specimens in the pathology suite; the SOP is posted on the website. These body fluids are currently collected only at JHU, but NYU will begin implementing the SOP for prostatic fluid.

5. Marketing and Usage

5a. Marketing. We distributed a 1 page flyer (similar to that used for the March 2011 IMPaCT meeting) to all participants at the Prostate Cancer Foundation meeting in September 2011. In January 2012 we sent out a pilot survey to prostate cancer scientists recently funded by the DOD (using an email list provided by Drs. Nrusingha Mishra and Carolyn Best). Surveys were sent to 105 researchers; a total of 9 people responded, but traffic at the website (209 new visits) went up 55% compared to the previous month. In February 2012 the Prostate Cancer Foundation graciously agreed to send our survey to researchers on their mailing list under Dr. Soule's signature. The survey was sent to 296 researchers representing a majority of the most active prostate cancer scientists and 84 responded; website traffic recorded 265 new visits. Some of the interesting survey results are as follows:

- 91% said their current needs for tissue were not met by available resources

- 89% were interested in primary tumor tissue
- >80% were interested in metastatic tissues
- nearly 80% were interested in TMAs with matched recurrent and non-recurrent cases
- 70-80% were interested in DNA and RNA
- >60% were interested in serum

Other survey responses are included in **Appendix 3**.

In February 2012 PCBN members also presented 2 posters at the 5th Annual Biospecimen Research Network Symposium in Bethesda:

“Ischemia/Fixation Trial Tissue Microarray”

“Biobanking as part of the Prostate Cancer Biorepository Network (PCBN): A focus on DNA, RNA and Protein Derivatives from Radical Prostatectomy Specimens”

In April 2012 the PCBN had a booth in the Exhibitors Hall at the AACR meeting in Chicago and presented information and literature on the PCBN. Traffic to the website increased 265% compared to March 2012.

In May 2012 the PCBN presented 2 posters at the ISBER Annual Meeting in Vancouver:

“The Prostate Cancer Biospecimen Network (PCBN)”

“Ischemia time monitoring: Experience of the Prostate Cancer Biorepository Network”

Abstracts for the BRN Symposium and ISBER Annual meeting are included as **Appendix 4**, and are also posted on the website.

Finally, in the most recent program announcement about opportunities for prostate cancer from the DOD PCRP, the PCBN was specifically highlighted and applicants were encouraged to request biospecimens from the PCBN (see next section).

5b. Usage of Biorepository. The following breakdown describes queries and requests received and responded to as of June 20, 2012:

Queries received and responded to: 31

Requests for specimens received: 15

Number of requests fulfilled and samples shipped: 6

Status of remaining 9 requests:

- approved & awaiting MTA response from requestor's institution 1
- reviewers requested additional clarification 4
- requests currently approved, samples in preparation for shipment 3

The requests are from 14 different investigators at the following institutions (all institutions had 1 request from 1 investigator except 5 requests from 4 investigators at Johns Hopkins):

Biological Dynamics, Inc.

Dana Farber

Johns Hopkins

National Cancer Institute

NYU

Thomas Jefferson University

University of Pennsylvania

University of Sydney, Australia

University of Wisconsin

Washington University

Weill Cornell Medical College

Some factors influencing time to complete a request:

- a. Inadequate information provided by requestor: To rectify this we have made our request forms much more explicit. We also “pre-qualify” requests when we receive initial queries by trying to determine their specific needs and telling them what information they’ll need to provide, before they fill out a request form.
- b. MTA review process: delays have been encountered at both JHU and NYU, and at requestor institutions. This is improving as the tech transfer offices at JHU and NYU get more familiar with the scope of PCBN activities.
- c. Systematic approach to handling queries, requests: since the “query” function from the website sends queries to the entire PCBN team we outlined a specific framework for responses and responsibilities at our May 2012 face-to-face meeting at NYU.
- d. Reviewers have taken some time to become accustomed to the criteria for specific sample types. We now assign a specimen priority to the request before sending to the reviewers.

In addition to formal applications for samples, we recently received 5 requests for **letters of support** from investigators who have received letters of invitation from the DOD to submit full grant applications in response to the 2012 PCRP RFA; we are in process of determining the requirements for each of these and preparing letters of support (one letter has already been sent to an investigator at the University of Bristol UK).

Addressing changes in key personnel

In March 2012 Dr. De Marzo accepted a position at a private sector firm. However, he retains a 20% appointment at Johns Hopkins for at least 1 year, and comes to Johns Hopkins weekly, still maintains a lab, and

meets regularly with Drs. Trock, Netto, Sfanos, and Ms. Fedor about PCBN activities. He has also been acquainting Dr. Tamara Lotan, an excellent uropathologist, with the activities and functions of PCBN. Thus, for at least 1 year his participation in PCBN is unchanged. At the end of the year he will re-assess his role at Johns Hopkins. If he no longer maintains an appointment at Johns Hopkins, Drs. Netto and Lotan will assume his remaining responsibilities.

James Morgan, the informatics specialist at JHU has also left, but is still providing support on an as-needed basis as part of the arrangement with Dr. De Marzo. We also have temporary support from the team responsible for implementing caTISSUE at JHU. We are also in discussion with the Chairman of Urology about recruiting a replacement (since Mr. Morgan's position on the grant only covered a portion of his effort).

Response to Previous EAB Review (September 7, 2011):

In addition to the issues briefly mentioned above, other key issues that we are addressing are as follows:

Define metrics to determine success: given the initial funding period of only 3 years, it is not likely that we will be able to assess clinical impact. However, we will evaluate scientific impact by the number of tissue requests that are filled, the number of grants that are supported by PCBN tissues, the number of publications or conference presentations based on PCBN tissues, the number of publications or conference presentations resulting from biospecimen science conducted by PCBN investigators, and the number of biomarkers evaluated in PCBN tissues. We are currently determining reasonable values for each of these parameters to serve as quantifiable metrics. We will also define metrics for success at 5 years, assuming that we will obtain additional funding from the DOD or other sources of support.

Collaborations: The biggest challenge to establishing collaborations whereby other investigators allow their biospecimens to be accessed through the PCBN concerns control over specimens. Because such specimens are typically acquired, with difficulty and usually considerable expense, to foster the research of the individual investigator or group of investigators, there is little incentive to provide them to outside users. As described in Section 5, we are in discussion with Drs. James Mohler and Carl Morrison to provide specimens from African American patients from the Prostate Cancer Project Consortium (PCPC) for our Race TMA. Some of the issues encountered have been cost recovery, joint ownership of samples, MTA, and requirement to be a collaborator on all PCPC samples shared by PCBN. We have also contacted Dr. Christopher Logothetis (MD Anderson Cancer Center) who has agreed to provide us with protocols and guidance for collection of bone marrow biopsies, but is not able to share their biopsy specimens. We are in discussion with oncologists at Johns Hopkins (Drs. Carducci and Eisenberger) about the possibility of implementing a bone marrow biopsy protocol at JHU but the biopsies are done by interventional radiology and require reimbursement. We have also been in contact with Dr. Howard Scher about collaborations with the DOD Clinical Trials Consortium. Although Dr. Scher does not think it will be feasible to provide us with biospecimens from trial participants he has proposed a collaboration where the PCBN would perform assay validation to determine the best assay for a biomarker target assessed in a targeted therapy trial. The PCBN will also be a collaborator in a large multi-center biomarker discovery and validation proposal that Dr. Scher is submitting for peer-reviewed funding. We have had successful discussions with Drs. Alan Partin and Mario Eisenberger at JHU, resulting in agreement to share urine samples collected by Dr. Partin, and a bank of serum samples from men with advanced disease collected by Dr. Eisenberger. Some of the challenges with those collaborations include IRB modifications and transferring databases. Dr. Ken Pienta has agreed to let us put a link on our website to their SPORE tissue core which also shares specimens.

Finally, Dr. Melamed is in discussion with several hospitals in NY about accruing metastatic and advanced disease tissue specimens from their pathology archives.

Workshop planning: We are developing a draft plan for a workshop to be held before the end of the funding period. The workshop is intended to include experts in biorepositories, biospecimen science, pathology, and translational research, and will focus on ways to improve biorepositories and specimens, establish linkages among existing repositories to facilitate access to tissues for the research community, and prepare for the yet to be determined biospecimen requirements of newer technologies/systems biology. A draft outline for the workshop is in **Appendix 5**.

KEY RESEARCH ACCOMPLISHMENTS

The collaboration between the DOD, NYU and JHU to establish the PCBN as a pilot program for a state of art prostate cancer biorepository now fully operational. The PCBN has achieved the following milestones:

- a. Establishment of governance, including prioritization and review policies (posted on website)
- b. Satisfaction of regulatory requirements
- c. Development of caTISSUE-accessible informatics infrastructure and CDEs
- d. Construction of website to serve as access point for users
- e. Establishment of a sizable collection of tissues in TMAs, and availability of body fluids; other TMAs and derived specimens (DNA/RNA)
- f. Development of harmonized SOPs (posted on website)
- g. Detailed quality control assessment of SOPs for DNA and RNA (posted on website).
- h. Evaluation of p27 immunohistochemistry as surrogate for fixation quality. This biospecimen science research will be enhanced by development of a Fixation TMA that will allow us to evaluate the impact of variation in fixation time and processing time.
- i. Survey sent to researchers on mailing lists of DOD PCRP and Prostate Cancer Foundation to raise awareness of PCBN and determine needs of community
- j. Poster presentations describing the PCBN and presenting biospecimen science (DNA and RNA quality; ischemia and fixation issues) at the DOD-sponsored Innovative Minds in Prostate Cancer Today (IMPaCT) meeting, Biospecimen Research Network Symposium, ISBER Annual Meeting, and a booth at AACR.
- k. Requests for biospecimens are now being received, reviewed, and fulfilled

REPORTABLE OUTCOMES

Abstracts and presentation: 2 presentations on the PCBN and its biospecimen science were presented at the 5th Annual Biospecimen Research Network Symposium in Bethesda, and 2 presentations were made at the International Society for Biological and Environmental Repositories (ISBER) Annual Meeting in Vancouver. Biospecimens have been accrued from 1169 prostate cancer patients in the last 12 months, and 3 new TMAs have been constructed, with 4 more currently in development.

A comprehensive website has been developed with forms for applying for specimens.

DNA and RNA quality control protocols have been optimized and the results of these experiments are being prepared for publication.

CONCLUSIONS

At the end of the 2nd year of funding the PCBN is fully operational, with established policies and procedures, SOPs, functioning informatics infrastructure, comprehensive website, large catalog of biospecimens, and usage from investigators around the world. We have 5 goals for the 3rd year. (1) Our primary goal is to increase usage. Now that the PCBN is fully operational we expect awareness of the resource to grow as more specimen requests are fulfilled. We will also continue to market the PCBN at research conferences and other venues. (2) In concert with this goal we intend to increase accrual of high demand samples, i.e. metastatic and high risk disease tissues, derivatives, serum matched to primary tissue, and specimens from African Americans. This also entails finishing several TMAs that are currently in development, notably completing the Race Disparity TMA with specimens from NYU and the Prostate Cancer Project Consortium, and the High Grade Recurrence TMA. (3) A major goal is to conduct the Workshop (see **Appendix 5**). We will be in contact with organizers of major conferences such as AACR or the AUA to see if the Workshop can be held in conjunction with a conference. One of the desired outcomes from this workshop will be a large-scale multi-center collaboration. (4) We plan to conduct biospecimen science studies to evaluate how variation in fixation parameters and ischemia affect a range of commonly used biomarkers, using our newly constructed Fixation and Ischemia TMAs. (5) Finally, we plan to write a manuscript describing the development of the PCBN and lessons learned.

The PCBN was conceived and funded as a pilot effort. It is well-positioned to fulfill that goal and, with additional funding (that will hopefully include at least one more Network Site), continue as a full-functioned biorepository. Sources for this funding are: (1) Institutional: discussions are underway with the Department of Urology at both institutions, as well as from the Cancer Center Support Grant at Johns Hopkins. (2) Foundation support: We have contacted Movember/Global Action Plan and will also seek support from the Prostate Cancer Foundation, other foundations, and individual donors identified by the development offices at both institutions. (3) Grant support: We will submit grants to the National Cancer Institute as well as the Department of Defense. If necessary, a 4th possibility would be to actively seek greater support from industry, although this is not our preference and we would like guidance from the EAB and the DOD about this.

APPENDICES

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OverviewVisits **vs.** Select a metric

Hourly Day Week Month

Visits

400

200

91 visits

135 visits

209 visits
Pilot survey sent out 1/31265 visits
Survey to PCF sent on 2/16
OBRR started 2/22347 visits
AACR started in 4/1228 visits
ISBER started 5/15

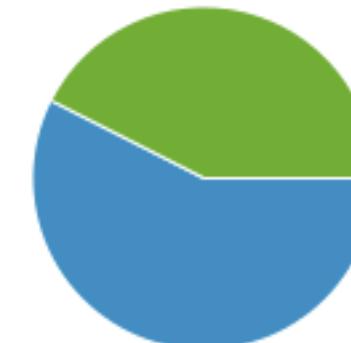
Dec 2011

Jan 2012

Feb 2012

Mar 2012

Apr 2012

823 people visited this site **Visits: 1,406** **Unique Visitors: 823** **Pageviews: 5,207** **Pages / Visit: 3.70** **Avg. Visit Duration: 00:02:48** **Bounce Rate: 46.02%** **% New Visits: 57.40%****57.47% New Visitor**

808 Visits

42.53% Returning Visitor

598 Visits

Due Diligence:
Selection of SOPs for DNA, RNA and protein extraction from frozen prostate tissues

Compiled By Karen Sfanos, Ph.D.
March 2012

The goal in the development of DNA, RNA and protein extraction SOPs was to choose the processing method that preserves the greatest number of analytes while maximizing quality/yield:

1. DNA

We used the following criteria in reviewing protocols for use for DNA extraction from frozen prostate tissues:

- 1) The method must provide DNA of adequate quality for advanced technologies (e.g., next generation sequencing, exome sequencing, methylation and other epigenetic studies, etc.)
- 2) The DNA should be treated with RNase as part of the protocol
- 3) The DNA samples must remain free of PCR inhibitors

We considered several different protocols for DNA extraction including phenol/chloroform extraction, the Qiagen AllPrep kit, and the Qiagen DNeasy Blood and Tissue kit (which is identical to the Qiagen QIAamp kit with the exception of the handbook, see [http://www.qiagen.com/faq/faqview.aspx?faqid=760&SearchText=&FaqCategoryId=0&MenuItemId=0&catalog=1&ProductLineId=0](http://www.qiagen.com/faq/faqview.aspx?faqid=760&SearchText=&FaqCategoryId=0&MenuItemID=0&catalog=1&ProductLineId=0)). We have tested these protocols side-by-side for use on frozen prostate tissues, and have found the Qiagen DNeasy Blood and Tissue kit gives the optimum yield of DNA. Studies conducted in our laboratory have verified that DNA extracted from frozen prostate tissues with the Qiagen DNeasy kit can be successfully used for real-time PCR experiments for 18S and β -globin. Furthermore, in previously published studies, the Qiagen DNeasy kit has been successfully used for extraction of DNA from frozen prostate tissues used for advanced technologies such as next-generation deep sequencing studies¹, exome sequencing studies² and bisulfite sequencing and DNA methylation studies^{1,3-5}.

2. RNA

We used the following criteria in reviewing protocols for use for RNA extraction from frozen prostate tissues:

- 1) The method must provide RNA of adequate quality for advanced technologies (e.g., microarray, RNA-seq, NanoString, etc.)
- 2) The method used must preserve small RNAs (microRNA, etc.)
- 3) The samples must remain free of protein, DNA carry-over, PCR inhibitors, etc.

Since our criteria for an RNA extraction protocol required that the method preserves small RNA species, this excluded all column-based RNA extraction protocols. Even so, we have previously compared column-based RNA extraction kits (such as the Qiagen RNeasy kit) to the Trizol Reagent (Life Technologies) procedure, and have found that Trizol maximizes RNA yield. Since Trizol preserves all RNA species in a sample, we opted for this method for RNA extraction. Studies conducted in our laboratory have verified that RNA samples extracted with Trizol from frozen prostate tissues routinely have RIN numbers above 7 (and often above 8). These Trizol-extracted RNA samples have been successfully used for real-time PCR experiments for 18S and GAPDH. Furthermore, real-time PCR experiments for Racemase and hepsin on tumor and normal prostate tissues pairs extracted with Trizol have shown the expected distribution of elevated Racemase and hepsin expression levels in prostate cancer tissues.

The Johns Hopkins microarray core facility (as well as many other microarray core facilities throughout the country) recommends the use of Trizol for RNA extraction from mammalian tissues for all microarray and, more recently, NanoString analyses. In previously published studies, RNA extracted from frozen prostate tissues using Trizol Reagent has been successfully used for advanced technologies such as microarray analyses⁶⁻⁸ and RNA-seq⁹.

3. Protein

We are currently developing SOPs for protein extraction from frozen prostate tissues. Our initial approach has been to compare protein extraction procedures side-by-side. Protocols that we are currently considering are extraction with a RIPA buffer that we have used for previous studies in the lab¹⁰⁻¹², and extraction with Cell Signaling products such as their RIPA buffer (Cat. #9806) and cell lysis buffer (Cat. #9803). We are evaluating total protein yield (BCA protein assay) and quality (by western blotting for proteins including androgen receptor (AR), cMYC, ERG and PTEN as well as phosphoproteins such as pAKT and pS6) in the comparison of these methods. These efforts are currently ongoing.

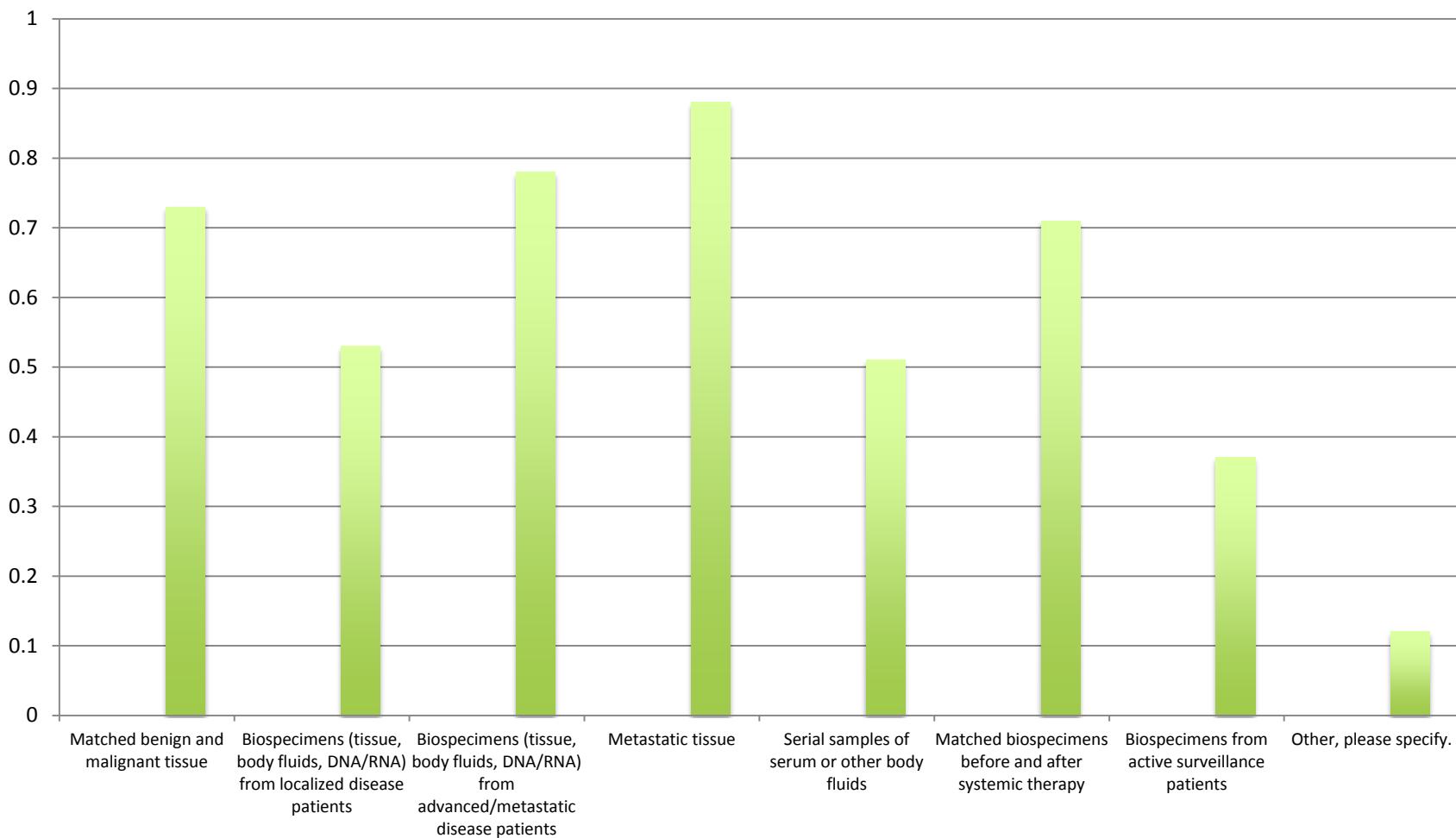
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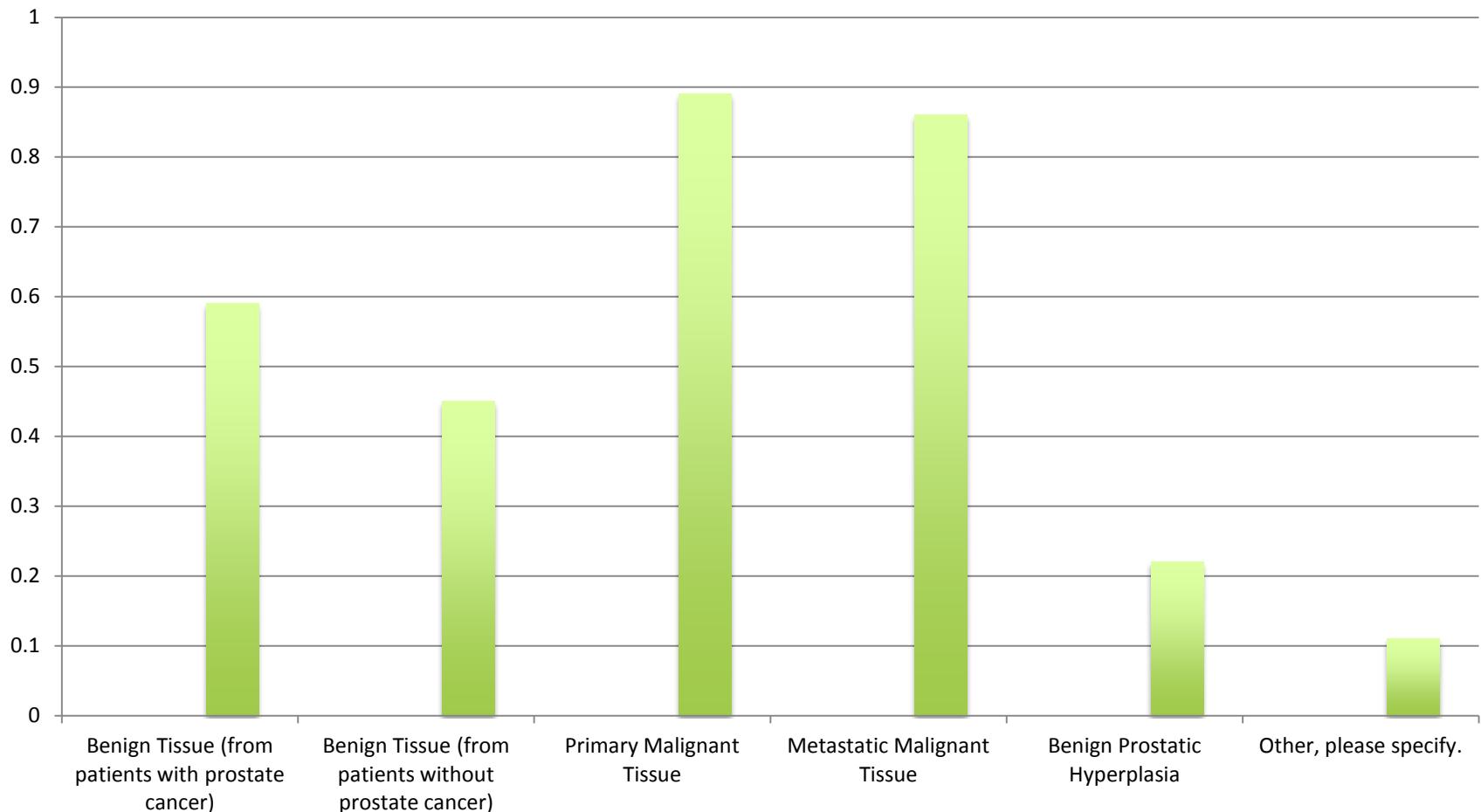
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Survey Results

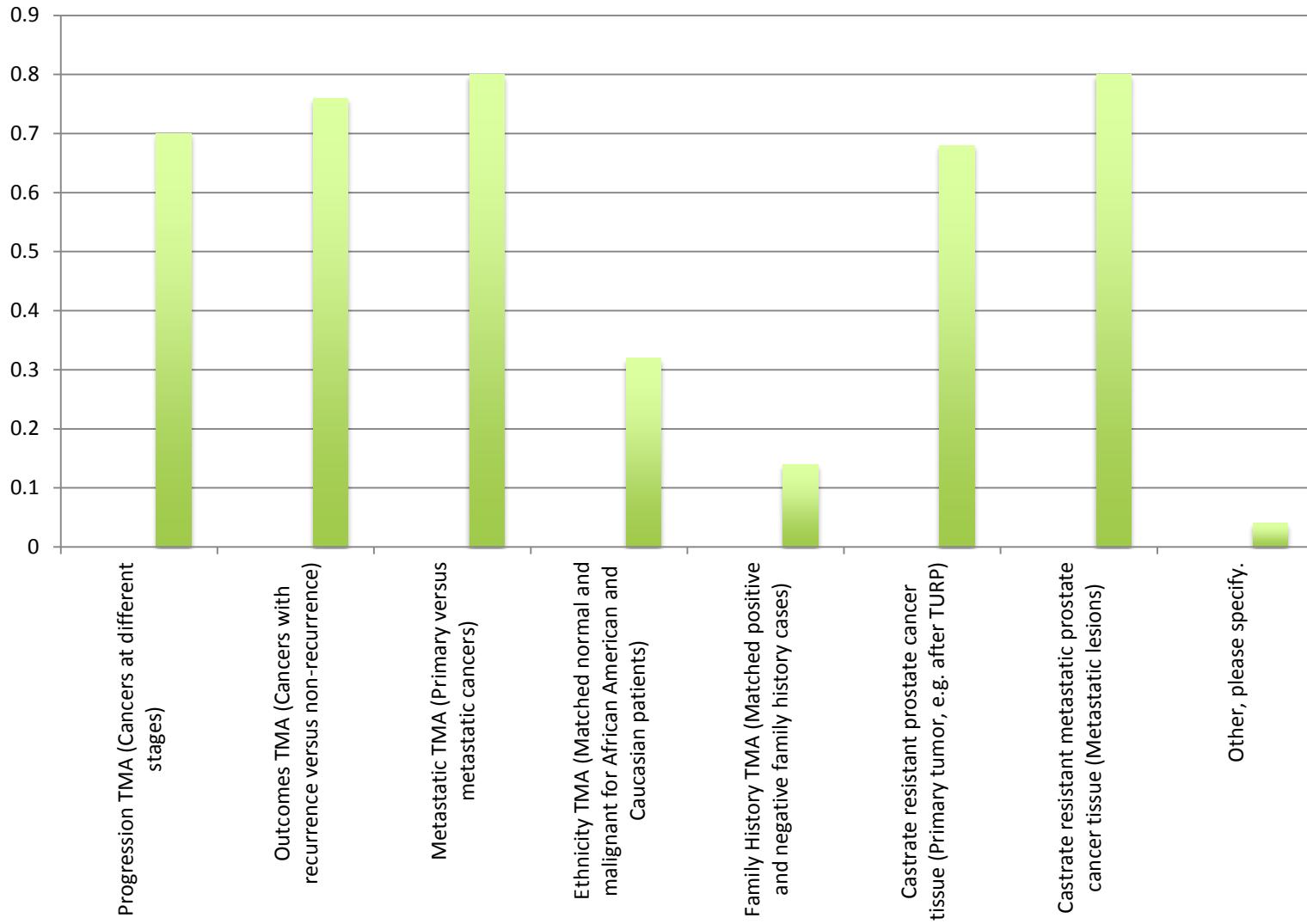
What types of specimens would be of greatest use to you if they were available from PCBN?(Note: Check all that apply.)



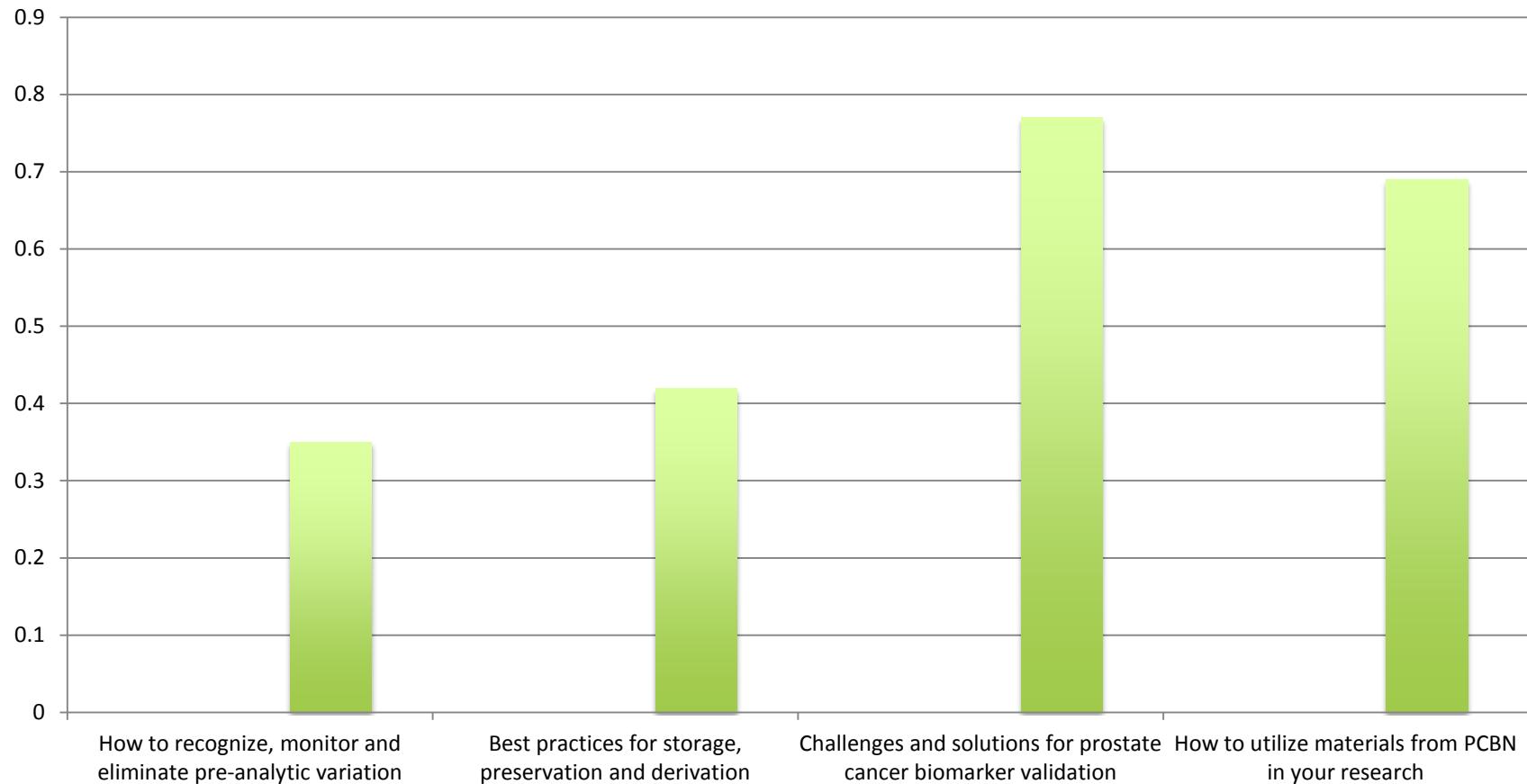
What types of samples do you normally require? (Note: Check all that apply)



What types of tissue microarrays (TMAs) would you be interested in?(Note: Check all that apply)



The PCBN will be holding a workshop in the next year. Which topics would be of interest to you?



Biospecimen Research Network Symposium

February 2012, Bethesda

BIOBANKING AS PART OF THE PROSTATE CANCER BIOREPOSITORY NETWORK (PCBN): A FOCUS ON DNA, RNA AND PROTEIN DERIVATIVES FROM RADICAL PROSTATECTOMY SPECIMENS

Medha Darshan¹, Helen L. Fedor¹, Qizhi Zheng¹, Patricia Kolmer², Ruth Pe Benito³, Monica Gorman³, Peng Lee^{3,4}, Jonathan Melamed³, Bruce J. Trock², Angelo M. De Marzo^{1,2}, Karen S. Sfanos¹

Departments of Pathology¹ and Urology², Johns Hopkins University School of Medicine, Baltimore, MD, Departments of Pathology³ and Urology⁴, New York University School of Medicine, New York, NY

The Prostate Cancer Biorepository Network (PCBN) is a collaboration between the Johns Hopkins University School of Medicine (JHU), the New York University School of Medicine (NYU), and the Department of Defense (DOD). The goal of the PCBN is to develop a biorepository with high-quality, well-annotated specimens obtained in a systematic, reproducible fashion using optimized and standardized protocols, and an infrastructure to facilitate the growth of the resource and its wide usage by the prostate cancer research community. One specific focus of the PCBN is to characterize critical parameters in the biospecimen “life cycle” that influence the molecular integrity of research tissues. We will describe our efforts to develop Standard Operating Procedures (SOPs) for the extraction and biobanking of DNA, RNA and protein derivatives from frozen tissues harvested from radical prostatectomy specimens. This involved side-by-side comparison of extraction methods and optimization for prostate tissues. We have also developed a series of Quality Control (QC) procedures which included establishing standardized methods for quantification and assessing sample quality. We have developed a routine series of real-time PCR assays for DNA and RNA samples based on both housekeeping genes (GAPDH, 18S, β -globin) and markers differentially expressed in prostate cancer (Racemase, hepsin) that are performed on all samples included in the biorepository. The aim of performing these assays is in assuring that the samples included in the PCBN are of sufficient quality for use in downstream applications. We will also plan to assess real-time PCR data in relation to pre-clinical variables such as warm ischemia time, time to tissue harvest, and age of the specimen. Additional efforts currently underway include determining global changes in RNA expression and protein quality in frozen tissues collected from open radical prostatectomy versus laparoscopic prostatectomy and the development of SOPs for DNA/RNA extraction from archival formalin-fixed paraffin-embedded prostate tissues.

ISCHEMIA/FIXATION TRIAL TISSUE MICROARRAY

Ruth Pe Benito^{1*}, Helen Fedor^{2*}, Monica Gorman¹, George Netto², Karen Sfanos², Patricia Kolmer², Medha Darshan², James Morgan², Peng Lee¹, Angelo De Marzo², Bruce J. Trock², Jonathan Melamed¹

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Since its introduction over 10 years ago, tissue microarray (TMA) technology has become an indispensable tool in biomedical research. Although there are clear benefits to their use in biomarker discovery, TMAs have some significant weaknesses. TMAs often use large cohorts of archival material from vast time spans, increasing the chance that variations introduced not only by the age of the archival tissue but also by diverse tissue handling and specimen preparation protocols can confound results. Pre-analytic variables, specifically fixation and ischemia, have been implicated as key variables in the measurement of proteins by immunohistochemistry (IHC). To better understand the influence of fixation length and ischemic time on the final IHC result, the Prostate Cancer Biorepository Network (PCBN) collected tissue samples from radical prostatectomy specimens (n=42) with known ischemic intervals. The tissue samples were divided into four 5-7mm punches for delayed intervals to fixation (0, 1, 2 and 4hrs) (n=27) or five 5-7mm punches for fixation in 10% neutral buffered formalin at varied time lengths (4, 8, 12, 24 and 48hrs) (n=15). Samples for delayed fixation were kept were placed in a moisture chamber to prevent them from drying out. Fixed samples were placed in PBS to prevent further cross-linking, prior to processing using a uniform protocol. A tissue microarray (TMA) will be constructed and assessed with known markers of ischemia, fixation and tissue quality (p27and phospho-antibodies). Further, as the magnitude of ischemic change and fixation will be known, this TMA will be useful for determining their effects on tissue quality and marker reactivity. It is planned to make this TMA available to researchers accessing PCBN for validating antibodies.

ISBER Annual Meeting

May 2012, Vancouver

ISCHEMIA TIME MONITORING: EXPERIENCE OF THE PROSTATE CANCER BIOREPOSITORY NETWORK

Ruth Pe Benito¹, Monica Gorman¹, George Netto², Karen Sfanos², Helen Fedor², Patricia Kolmer², Medha Darshan², James Morgan², Peng Lee¹, Angelo De Marzo², Bruce J. Trock², Jonathan Melamed¹

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Background

Translational research projects have increasingly relied on the use of high-quality, well-annotated and well-characterized biospecimens. Although ample biospecimens are available, they often represent convenience samples resulting in potentially incomparable populations. For this reason, tracing pre-analytical variables that potentially affect quality and comparability are imperative in ensuring experimental differences is attributed to the pathological condition rather than a biological response to environmental changes and biological stresses introduced by biobanking. A well-known contributor of variation is ischemia. Conventionally, 30-minutes before preservation is considered the limit for conservative treatment, however, it is difficult to accept that it only takes 30-minutes between time-of-devascularization of a robotic–prostatectomy specimen and time-of-preservation.

Methods

We collected precise ischemia times for robotic-prostatectomy specimens in the setting of a tertiary-care hospital. With the assistance of surgical/OR staff, we monitored precise devascularization times in addition to acquisition, processing and preservation to calculate the total time these tissues are subjected to ischemic conditions.

Results

We found an increase in acquisition time over estimates (30mins to 2.5hr). Furthermore, we confirmed that specimens remain at 37C, with limited-to-no blood supply for approximately 1.5hr.

Conclusions

Inaccurate estimates in acquisition times of biospecimens could potentially be detrimental to downstream applications. Research has shown resulting changes in expression profile both at the mRNA and the protein level, and has been reported in as little as 5-minutes following tissue excision. Therefore, given the under-estimation of acquisition time, standardizing protocols where possible, and precise tracing of pre-analytical variables are imperative in minimizing and accounting for experimental variance.

THE PROSTATE CANCER BIOSPECIMEN NETWORK (PCBN)

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Background

Recent acceleration on new technological platforms increased demands on biospecimens used for post-genomics research projects. This coincided with a shift in the banking of biospecimens as variability can be attributed to processing history rather than intrinsic differences, resulting in limited availability of biospecimens useful optimally for research. The Prostate Cancer Biorepository Network (PCBN), a collaboration between Johns Hopkins School of Medicine (JHU), New York University School of Medicine (NYU), and Department of Defense (DOD), was developed in recognition of this need. Although prostate cancer (PCa) biospecimens are available at many institutions, they often represent convenience samples, lack detailed annotation and are collected and processed without uniform protocols.

Method

PCBN procures clinically-annotated fresh-frozen and formalin-fixed prostate tissues, fluids and derived analytes, in a systematic, reproducible fashion under stringent conditions. PCBN conducts biospecimen science research to annotate critical parameters in the biospecimen “life cycle” and evaluate their impact on molecular integrity and biomarker findings.

Results

The biospecimens offered include large, comprehensively-annotated cohorts that accurately represent the spectrum of PCa. Tissue microarrays (TMAs) are constructed for rapid biomarker discovery studies and verified for adequate fixation. Analytes are derived with maximal recovery from samples with known hypoxic and thermal histories to ensure comparable molecular profiles.

Conclusions

Clinical translation of promising biomarker research is hampered by lack of availability of high-quality, well characterized prostate specimens and lack of understanding of the impact of pre-analytical variation on biomarker test results. The PCBN will provide critical resources and biospecimen science to enhance the validity and translation of PCa biomarker research.

Draft Outline for PCBN-sponsored Workshop

Workshop title: Analytical challenges in biomarker translation

Venue: mini-symposium ideally held in conjunction with AACR 2013
(is joint sponsorship by AACR & DOD a possibility?)

Agenda:

Session 1 (morning): Quantifying the impact of pre-analytical variation

This session will (1) present data to characterize sources of pre-analytical variation with the greatest impact on translationally relevant biomarkers, and (2) identify biospecimens, bioassays, and biomarkers that are relatively more *tolerant* of such variation.

A goal of this session will be to produce a white paper, and also to determine interest in larger-scale multi-institutional collaborations to rigorously evaluate the impact of pre-analytical variation on a set of key prostate biomarkers.

Session 2 (afternoon): Validating biomarkers from discovery through translation

This session will focus on (1) what are the steps needed to take a promising new biomarker to the point where it can be used in meaningful translational studies, (2) what are the elements for valid studies utilizing TMAs (including the biorepository/biospecimen best practices needed to ensure rigorous TMA construction), and (3) biospecimen and bioassay criteria for promising new technologies (e.g. next-gen sequencing, RNA-seq, nanosensors, etc.).